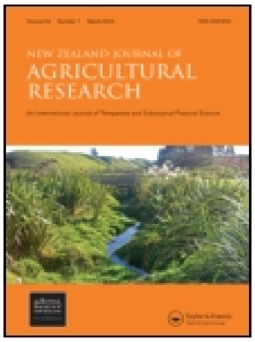

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Endophytic fungi affect growth of perennial ryegrass

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Abstract Clones of *Lolium perenne* L. 'Grasslands Nui' perennial ryegrass, either infected with or free from *Acremonium loliae* Latch, Christensen & Samuels, and clones of 'Grasslands Ruanui' perennial ryegrass, either infected with or free from a *Gliocladium*-like endophyte, were grown at 20°C for 8 weeks in a controlled-environment room. Nui plants infected with *A. loliae* yielded 38% more total dry matter than uninfected plants. Infected plants showed significant increases in total leaf area, tiller numbers, and growth of leaves, pseudostems, and roots, but the shoot : root ratio was unaffected. Infection of Ruanui with the *Gliocladium*-like sp. had no significant effect on yield even though total leaf area was reduced by 19%. The benefits in yield and protection from damage by Argentine stem weevil from *A. loliae*-infected ryegrasses are discussed in relation to the known risk of ill-health in animals grazing infected pastures.

Keywords *Acremonium loliae; Gliocladium*-like sp.; fungi; endophytes; *Lolium perenne*; perennial ryegrass; herbage yield; pasture production; tillering; ryegrass staggers syndrome; Argentine stem weevil

INTRODUCTION

Fungi have been observed growing intercellularly in several species of grasses (McLennan 1920; Sampson 1935; Neill 1941). Such fungi appeared to complete most, if not all, their life cycle within the plant, so they were commonly called endophytes. Endophytes of ryegrass (*Lolium* spp.) have been associated with the stock disorder, ryegrass staggers syndrome (Fletcher & Harvey 1981), and with protection of plants from damage by Argentine stem weevil (*Listronotus bonariensis* Kuschel) (Prestidge et al. 1982). Endophytic fungi appear to be exclusively seed-transmitted in *Lolium*, thus giving the option of establishing either endophyte-infected (infected) or endophyte-free (uninfected) perennial ryegrass (*Lolium perenne* L.) pastures.

Little information is available on the effect of grass endophytes on plant growth. Neill (1941, 1952) compared herbage yields from single plants of infected and uninfected perennial ryegrass in the field. Total herbage was measured just before flowering but no significant difference between infected and uninfected plants could be detected. Claims have been made that herbage yields from other grass species are affected by infection with endophytes, but little or no experimental data have been provided to verify them. Freeman (1903) stated that infected plants of darnel (*Lolium temulentum* L.) were more vigorous and productive than uninfected plants, but Sampson (1935) was unable to substantiate this claim. Harberd (1961) studied populations of *Festuca rubra* L. *sens. lat.*, many of which were infected with the endophyte, *Epichloe typhina* (Pers.) Tul. As some particularly lush plants were infected, he speculated that endophyte infection could cause increased plant vigour. However, Bradshaw (1959) found that *Agrostis tenuis* Sibth. and *A. stolonifera* L. plants infected with *E. typhina* produced no greater weight of herbage than uninfected plants, even though they formed more tillers.

Two endophytes — *Acremonium loliae* Latch, Christensen & Samuels (a fungus with asexual spores similar to those of *E. typhina*) and a *Gliocladium*-like sp. — have been found in New Zealand ryegrasses (Latch et al. 1984). This paper describes an experiment to determine the effect of these 2 endophytes on the growth of perennial ryegrass.

METHODS

Four 'Grasslands Nui' perennial ryegrass plants, naturally infected with *A. loliae* endophyte, were propagated clonally, and the endophyte was killed in some plants of each clone by growing tillers in a medium containing 200 ppm benomyl for 6 weeks (Latch & Christensen 1982). Thus for each genetically identical clone, plants were produced with and without *A. loliae* infection.

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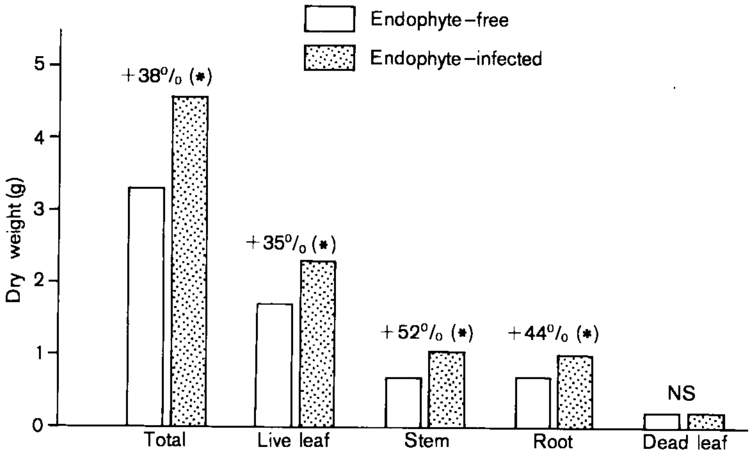

Fig. 1 Dry weights of *A. loliae*-infected and uninfected Nui ryegrass plants after 8 weeks growth at 20°C. Percentage differences between treatments (endophyte-free = 100) shown above bars.

Four endophyte-free 'Grasslands Ruanui' perennial ryegrass seedlings were infected with the *Gliocladium*-like endophyte (unpublished data). Benomyl was used to eradicate endophyte in some plants of each clone so that genetically identical plant material was produced with and without the *Gliocladium*-like endophyte.

After the benomyl treatment, all plants were grown in a greenhouse for at least 12 months before use in this trial to allow fungicide effects to disappear.

Sterilised sand and non-sterile field soil (Kairanga silt loam), both amended with 1.5 g/litre 'Osmocote' slow release fertiliser (N, P, K ratio, 14 : 1.6 : 11.6), were used as growth media.

For each treatment, one uniformly-sized tiller was planted into each of six 10 cm pots of each growth media. After 10 days growth in a greenhouse (12–25°C) the 5 most uniform plants in each medium were selected (160 pots in total) and placed in a controlled-environment room. Nutrient solution (type O.N.C., Brooking 1976) was applied to each pot daily. The room was maintained at a constant temperature of 20 ± 0.5°C, a humidity of 5 millibars vapour pressure deficit (78 ± 5% RH), and a CO_2 level of 320 ± 30 ppm. Lighting was provided by four 1000 W Sylvania 'Metalarc' high pressure discharge lamps and four 1000 W Philips tungsten iodide lamps for a 12-h photoperiod. Mean photosynthetic irradiance at plant height was 131 Wm^2 (Li-190 SE flat response sensor) and mean photosynthetic photon flux density was 617 μmol m^{-2} s^{-1} (Li-190 S quantum sensor).

All plants were harvested after 8 weeks growth in the room. The leaf area of samples from each plant was measured with a Li-Cor 3100 area meter and the total leaf area was calculated. Numbers of tillers and dry weights of live leaves, dead leaves, pseudostems, and roots were recorded. Results were interpreted using factorial analysis of variance, accepting statistical significance at $P < 0.05$ unless otherwise indicated.

The presence or absence of the *Gliocladium*-like sp. in infected and uninfected Ruanui plants was confirmed by microscope examination before tillers were prepared for planting. The presence or absence of *A. loliae* in the harvested leaf samples from Nui was checked using the enzyme-linked immunosorbent assay (ELISA) method of Musgrave (1984), and mycelial concentrations were calculated from experimentally determined regressions of log ELISA index v. log mycelial concentration.

RESULTS

ELISA confirmed the presence of *A. loliae* in all leaf samples assumed to be infected, and the absence of *A. loliae* in all samples assumed uninfected. The mean concentration of *A. loliae* in leaf samples of the 4 infected genotypes was 0.62, 0.43, 0.35, and 0.18 µg/mg. These concentrations are considered to be high, and were significantly different between some genotypes ($0.62 > 0.35$ and 0.18).

Ruanui plants grown in sand had greater yields of pseudostem and dead leaf, greater total plant weight, and greater total leaf area than Ruanui plants grown in soil, but there was no interaction between the effect of endophyte infection and growth medium. Nui plants growing in sand had more dead leaf than Nui plants growing in soil ($P < 0.001$) but there was no interaction between the effect of endophyte infection and growth medium. All data presented are means from both growth media.

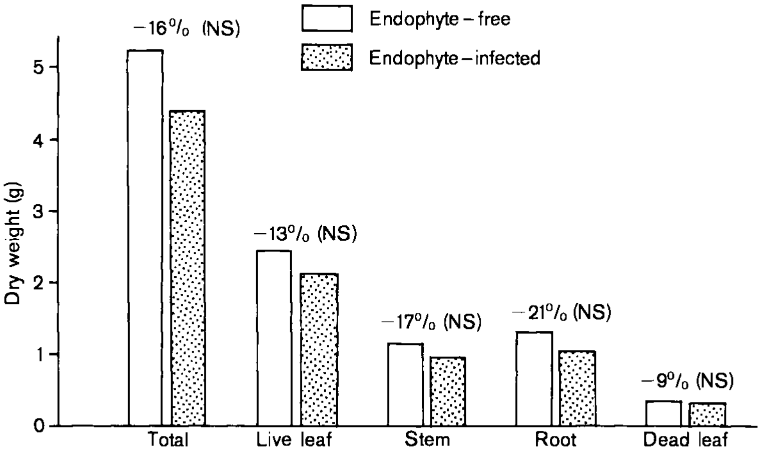

Fig. 2 Dry weights of *Gliocladium*-like sp.-infected and uninfected Ruanui ryegrass plants after 8 weeks growth at 20°C. Percentage differences between treatments (endophyte-free = 100) shown above bars.

Nui ryegrass infected with *A. loliae* had greater yields of leaf, pseudostem, and root than uninfected plants (Fig. 1). The mean total leaf area of infected plants (469 cm^2) was significantly greater than the total leaf area of uninfected plants (375 cm^2). The mean number of tillers per plant was also significantly greater in infected plants (28.1) than in uninfected plants (21.3).

Ruanui plants infected with the *Gliocladium*-like sp. had 19% ($P < 0.05$) less leaf area (425 cm^2) compared with uninfected plants (527 cm^2) but no significant difference was detected for leaf, pseudostem, root, or total yield (Fig. 2). There were also no significant differences in either the mean number of tillers produced by infected (50.2) and uninfected (49.8) plants, or in mean specific leaf area of infected (196 cm^2/g) and uninfected (209 cm^2/g) plants.

DISCUSSION

The experimental conditions for this trial were near the optimum for ryegrass growth (Hunt & Halligan 1981). Optimal conditions for growth of endophytes in plants are now known, but 20°C is close to the optimum temperature for growth of both fungi in axenic culture. Therefore, it is unlikely that there were environmental restraints to the expression of plant × endophyte interactions.

The results show that infection of Nui perennial ryegrass with *A. loliae* increased total dry matter 38% compared with uninfected plants. However, infection with *Gliocladium*-like sp. may have depressed leaf production by Ruanui, for although yield and tiller number differences were not significant, infection did reduce the total leaf area.

Neill (1941, 1952) was unable to detect any effect of endophyte infection on the yield of spaced plants in the field. Herbage yield of plants ranged from 3 to 550 g/plant, and failure to account for variation between plant genotypes could explain his inability to detect any influence of infection on plant growth. Variation between genotypes in our trial was significant ($P < 0.001$) for all measured parameters. However, in our statistical analyses the effect of endophyte infection could be separated from the effect of plant genotype because we used clonal material. We do not know which endophyte(s) were present in Neill's infected plants but our results indicate that only *A. loliae* has a large effect on plant growth.

The increased yield from *A. loliae*-infected ryegrasses was unexpected. Fungal infection can often result in reduced plant growth but a few fungi produce growth regulatory substances which increase the development of particular plant parts. One such fungus is *Gibberella fujikuroi* (Saw.) Wollenw., a pathogen of Gramineae, which produces gibberellin. This chemical growth regulator stimulates cell expansion so that infected plants may become slender and weakened (Ou 1972). Nui ryegrass infected with *A. loliae* had increased growth of all measured plant parts so there was no effect of infection on the shoot : root ratio or the live : dead ratio. The plants appeared normal, indicating that the relationship between fungus and host is probably symbiotic.

The magnitude of the differences in growth between *A. loliae*-infected perennial ryegrass and genetically identical uninfected plants has obvious implications for plant breeding. Past efforts to obtain genetically superior plants may have confused the effects of genetic differences and variation in endophyte infection. Such confusion may be aggravated by poor seed storage conditions which affect endophyte viability (Latch & Christensen 1982). The increased yield of *A. loliae*-infected ryegrass may be further amplified in the field because infected plants are resistant to attack by Argentine stem weevil (Gaynor & Hunt 1983). However, as endophytes have been associated with ryegrass staggers syndrome, it would be premature to advocate the use of *A. loliae*-infected ryegrasses until their full effects on animal health and performance are known.

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